Phytopathologia Mediterranea (2013) 52, 1, 157–165

RESEARCH PAPER

Combining Ascochyta blight and Botrytis grey mould resistance in chickpea through interspecific hybridization

Livinder KAUR[1], Asmita SIRARI[1], Dinesh KUMAR[1], Jeet SINGH SANDHU[2], Sarvjeet SINGH[1], Karan KAPOOR[1], Inderjit SINGH[1], C.L. Laxmipati GOWDA[3], Suresh PANDE[3], Pooran GAUR[3], Mamta SHARMA[3], Muhammad IMTIAZ[4] and Kadambot H.M. SIDDIQUE [5]

[1] Punjab Agricultural University (PAU), Ludhiana 141004, Punjab, India
[2] Indian Council of Agricutural Research, New Delhi 110114, India
[3] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India
[4] International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria
[5] The UWA Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

Summary. Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Labr. and Botrytis grey mould (BGM) caused by *Botrytis cinerea* (Pers. ex Fr.) are important diseases of the aerial plant parts of chickpea in most chickpea growing areas of the world. Although conventional approaches have contributed to reducing disease, the use of new technologies is expected to further reduce losses through these biotic stresses. Reliable screening techniques were developed: 'field screening technique' for adult plant screening, 'cloth chamber technique' and 'growth chamber technique' for the study of races of the pathogen and for segregating generations. Furthermore, the 'cut twig technique' for interspecific population for AB and BGM resistance was developed. For introgression of high levels of AB and BGM resistance in cultivated chickpea from wild relatives, accessions of seven annual wild *Cicer* spp. were evaluated and identified: *C. judaicum* accessions 185, ILWC 95 and ILWC 61, *C. pinnatifidum* accessions 188, 199 and ILWC 212 as potential donors. *C. pinnatifidum* accession188 was crossed with ICCV 96030 and 62 F_9 lines resistant to AB and BGM were derived. Of the derived lines, several are being evaluated for agronomic traits and yield parameters while four lines, GL 29029, GL29206, GL29212, GL29081 possessing high degree of resistance were crossed with susceptible high yielding cultivars BG 256 to improve resistance and to undertake molecular studies. Genotyping of F_2 populations with SSR markers from the chickpea genome was done to identify markers potentially linked with AB and BGM resistance genes. In preliminary studies, of 120 SSR markers used, six (Ta 2, Ta 110, Ta 139, CaSTMS 7, CaSTMS 24 and Tr 29) were identified with polymorphic bands between resistant derivative lines and the susceptible parent. The study shows that wild species of *Cicer* are the valuable gene pools of resistance to AB and BGM. The resistant derivative lines generated here can serve as good pre-breeding material and markers identified can assist in marker assisted selection for resistance breeding.

Key words: *Cicer arietinum*, *Cicer pinnatifidum*, interspecific hybridization, resistance, SSR markers.

Introduction

Chickpea (*Cicer arietinum* L.) is considered to be the earliest grain crop cultivated by man dating back to 7500–6800 BC as evident from the Middle Eastern archaeological sites (Zohary and Hopf, 2000). It has limited genetic diversity probably due to selection, addressing local problems associated with its cultivation, shift of cultivation from spring to winter or shift in area from cool winter to comparatively milder winter climates. The most important factors which can be linked to this low genetic diversity are the restricted distribution of wild progenitors, crossability barriers and linkage drag (Kumar *et al*., 2010). Another factor for low genetic diversity is linked to its monophyletic descent from the wild species *C. re-*

Corresponding author: L. Kaur
Fax: +91 161 2409891
E-mail: livinderk@rediffmail.com

ISSN (print): 0031-9465
ISSN (online): 1593-2095

ticulatum (Abbo *et al*., 2003). Recent research efforts have been oriented towards understanding evolutionary relationships within the genus *Cicer*, and that of wild *Cicer* spp. to *C. arietinum* based on biochemical aspects (Choumane *et al*., 2000; Gargav and Gaur, 2001) and DNA sequences (Choumane *et al*., 2000). Though systematic breeding work was initiated in 1960 through conventional breeding methods involving intraspecific hybridization, a remarkable gain in chickpea production and productivity could not be achieved as evident from the production (total production/total area) and productivity (kg ha^{-1}) statistics. Though there has been a productivity increase of approximately 33% from 1960–1961 to 2010–2011 (Singh, 2012), this is not comparable to wheat which experienced yield increases of approximately 269% during the same period. The genetic improvement through intraspecific hybridization has only led to genetic variability rather than genetic diversity for a particular trait because few parents with selected traits were repeatedly utilized in crossing programmes (Kumar *et al*., 2004). For example, 41% of chickpea genotypes have been developed where one or two parents were common and these developed genotypes were further used in crosses among themselves at one point or another. Another factor which determines the performance of a genotype is genotype × environment (G × E) interactions. It has been seen that G × E interaction in chickpea contributes 17–27% of the variability, however, the environment alone has 70–80% influence which may include the climatic factors, biotic and abiotic stresses. Among the biotic stresses the major is Ascochyta blight (causal agent *Ascochyta rabiei*) (AB) prevalent in countries where chickpea is grown in cool wet climate, and Botrytis grey mould (*Botrytis cinerea*) (BGM) in warm humid areas (Haware, 1998). As a high level of resistance is not available in cultivated chickpea, there is a need to develop genotypes with wide adaptability to various types of stresses and wild *Cicer* spp. appears to hold this promise. To harvest the maximum benefit from the wild *Cicer* spp., the relationship of wild species among themselves and the extent of diversity within collected accessions of a wild species need to be fully known. More recently DNA-based techniques have provided new tools to compare the genetic variability in terms of genomic variability. These techniques have given a boost to the conventional breeding methods. The hybrid nature of interspecific cross can be confirmed through use of molecular markers, and this technique is particularly useful where resistance is governed by recessive genes, minor genes or QTLs. In the present investigation, wild *Cicer* spp. were evaluated for resistance to AB and BGM which are the two major diseases of chickpea in the north western plain zone of India. Further, one accession of *C. pinnatifidum* (188) identified with high level of resistance to AB and BGM was used in interspecific hybridization to develop AB and BGM resistant derivative lines.

Materials and methods

Identification of AB and BGM resistance in wild *Cicer* species

Material included different accessions of annual wild *Cicer* species, viz. *C. judaicum, C. pinnatifidum, C. reticulatum C. yamashitae C. echinospermum, C. cuneatum,* and *C. bijugum* screened using standard screening techniques for reliable results under controlled conditions against AB and BGM (Gurha *et al*., 2003). For AB screening, 'cloth chamber technique' was used while for BGM, 'growth chamber technique' was applied. Five seeds were sown in each pot for each accession in two replications arranged in randomized design with one pot per replication.

Multiplication of pathogens for inoculations

For AB screening, isolate 8, race 3968 (Singh, 1990) and for BGM screening, isolate 24, race 510 (Singh and Bhan, 1986) were used. These isolates were maintained on PDA (200 g potato, 20 g dextrose, 20 g agar, 1 L H_2O) slants and multiplied on Potato dextrose broth (500 g potato, 20 g dextrose, 1 L H_2O) at 22°C (for AB) and 25°C (for BGM) for inoculations.

Cloth chamber screening technique

Test material was sown in polyethylene pots (15 × 10 cm) filled with sandy-loam soil, 5 seeds per pot in the glass house maintained at 25–28°C. C214, a susceptible variety was used as check to monitor the disease epidemic. Twenty-five polyethylene pots containing 20–25 days old seedlings of the test material were stacked along with a pot of check in 8–10 cm deep pit of 25 cm diameter. Water was added in and around the pots in the morning and evening before inoculation. Inoculation was done by spraying a spore suspension of *A. rabiei* at a concentration of 4×10^4 spore mL^{-1}. These pots were then covered by an iron cage over which a moist double thread knitted

cotton cloth ('*Dasuti*') was draped. The bottom of the cage was buried in the soil to reduce any free passage of air into these chambers. High humidity was maintained at 85% RH and an ambient temperature of 21–22ºC during day time and 15–16ºC at night in these chambers for a period of 48 h of incubation by spraying water at 1.5 h interval during day time with knap sac spray pump operated manually. After the incubation period, chambers were removed, but spraying of water was continued up to 13 days during day time at 1.5 h intervals between 10:00 h to 16:00 h. The disease symptoms started appearing 7 days after inoculation and observations were recorded 13 days after inoculation on a 1–9 scale (Table 1) (Gurha *et al*., 2003).

Growth chamber screening technique

Five seeds of each test lines were sown in polyethylene pots (15 × 10 cm) containing sandy-loam soil and maintained in a glass house. One month old plants of the test lines along with the susceptible check G543 were transferred to growth chambers, watered and inoculated with a spore suspension containing 1×10^4 spore mL^{-1}. These plants were kept in moist chambers (polyethylene bags supported by iron cage) for 144 h with 8 h dark and 16 h light periods provided through a fluorescent lamp (24" × 1.5", W 20, 32 lm/W.). Observations on disease incidence were recorded on a 1–9 point rating scale (Gurha *et al*., 2003) (Table 1) after 6 days of inoculation.

Development and evaluation of interspecific hybrids

Wild *Cicer* species *C. pinnatifidum* accession 188 which was identified for possessing resistance to both, AB and BGM was used in interspecific hybridization with *C. arietinum* ICCV 96030. To obtain higher seed setting and fertile F_1 plants, a growth hormone solution containing 75 ppm GA_3 + 1 ppm NAA + 10 ppm Kinetin was applied on flower peduncle soon after pollination. During 2000–2001, fertile F_1 hybrids from the above cross were advanced to the F_9 generation through the pedigree method, totaling 193 lines designated as derivative lines identified with the pre-

Table 1. Disease rating scale for Ascochyta blight (AB) and Botrytis grey mould (BGM) in chickpea using different screening techniques.

Scale	AB	BGM		Disease Response
	Field and cloth chamber technique	Growth chamber technique	Cut twig technique	
1	No disease visible on any plant	No infection on any part of plant	No visible symptoms on any part of twig	Highly resistant [HR]
3	Lesions visible on less than 10% of the plants, no stem girdling	Minute water-soaked lesions on 5% leaves	1–2 lesions on leaves	Resistant [R]
5	Lesions visible on up to 25% plants, stem girdling on less than 10% plants but little damage	Lesions and soft rotting on 11–25% of leaves and tender shoots	1–2 leaves give burnt appearance, slight stem soft rotting	Moderately resistant [MR]
7	Lesions present on most of the plants, stem girdling on 50% of the plants and resulting in death of a few plants causing considerable damage	Rotting and fungal growth on 41–55% of leaves and shoots	Soft rotting of stem 50% foliage killed	Moderately susceptible [MS]
9	Lesions profuse on all plants, stem girdling present on more than 50% of plants and death of most plants	Extensive rotting and fungal growth on 71–100% of leaves, shoots and stem	Extensive soft rotting of stem and foliage with fungal growth on foliage, whole twig killed	Susceptible [S]

fix 'GL' for further use in breeding programs or for the development of a mapping population. The set of 193 derivative lines was evaluated for AB and BGM resistance during 2008–2009 using 'field screening technique' for AB and 'cut twig technique' for BGM resistance. Of 193, 62 lines with good growth habit and resistance to AB and BGM were re-evaluated for two consecutive years (2009–2010 and 2010–2011). Four resistant lines with combined resistance for AB and BGM, namely, GL 29029, GL29081, GL29206 and GL29212 were crossed with AB and BGM susceptible cultivar BG 256 to develop populations for mapping of AB and BGM resistance genes.

Field screening technique

In this screening technique, test lines were grown in 2 m rows, 40 cm apart. Susceptible varieties L550 and C214 were planted as indicator-cum-spreader rows after every 8 test rows. To establish uniform disease for AB screening, all plants of the test entries were spray-inoculated with conidial suspension of *A. rabiei* at 4×10^4 mL^{-1} in the evening at around 17:00 h in the first week of February each year when the crop was at the flowering and pod initiation stage, i.e. 85 to 90 days after seeding. Water sprinkling was done with sprinkler system operated by 7 H power diesel engine from day following inoculation for 10 min at 2 h interval from 10:00 to 16:00 h to maintain > 85% RH for 21 days. Disease symptoms started to appear 10–11 days after inoculation. Observations were recorded on a 1–9 rating scale (Gurha *et al*., 2003) (Table 1) during the last week of March.

Cut twig screening technique

In the cut twig method, the tender shoots of the chickpea plants were cut in tray containing water, immediately wrapped in wet cotton plug and placed into a test tube (15 × 100 mm) containing fresh tap water. Three twigs were tested from each wild accession or derivative line. Twigs were inoculated by spraying spore suspension of *B. cinerea* (10,000 spores mL^{-1}) and covered with moist polythene covers. Incubation and rating was done as described for the growth chamber screening technique. Disease observations were recorded at 1–9 scale, given in Table 1.

Identification of *C. pinnatifidum* specific segments

Total DNA was extracted from young leaflets of a single plant of *C. pinnatifidum* 188, ICCV 96030, GL 29029, GL 29206, GL 29212, GL 29081 and BG 256, using the CTAB extraction method (Saghai-Maroof *et al*., 1984). One hundred and twenty primer pairs as described by Winter *et al.* (1999), Choumane *et al.* (2000) and Nayak *et al*. (2010) were used in this study. PCR was performed in an Eppendorf thermocycler. Reaction mixture of 20 μL contained 60 ng DNA, 100 μM of each dNTP, 1.5 mM $MgCl_2$, 45 pmol of each primer and 0.2 U Taq DNA polymerase (Geneaid Biotech Ltd., Taiwan). Initial denaturation of DNA at 94°C for 4 minutes was followed by 35 cycles of denaturation at 94°C (2 min), annealing of primers at 55°C (50 s) and extension at 60°C (50 s). After a final extension at 60°C for 20 min, amplification products along with a 100 bp DNA ladder were separated on 2% agarose gels in TBE buffer. The DNA was stained with ethidium bromide and bands documented using gel documentation system (Alpha Innotech Corp., San Leandro, CA, USA). *C. pinnatifidum* specific bands were scored in the four derivative lines as present or absent. Likewise, polymorphism between derivative lines and BG 256 was also scored by estimating the allele size using the 100 bp ladder.

Results

Identification of AB and BGM resistance in wild *Cicer* species

The screening results for AB and BGM resistance of the accessions of 7 annual wild species, *C. judaicum*, *C. pinnatifidum*, *C bijugum, C. reticulatum*, *C. yamashitae*, *C. echinospermum* and *C. cuneatum* are presented in Table 2. In *C. judaicum*, three out of eight accessions, in *C. pinnatifidum* three out of five accessions and in *C bijugum* one out of the five accessions displayed resistant reactions to AB and BGM. Most of the accessions of *C. judaicum* showed high level of resistance to AB, whereas in *C. pinnatifidum* all the accessions showed resistance to BGM. One accession of *C. bijugum* (ILWC7/S-3) and two accessions of C. *echinospermum* (ILWC39 and 35/S-1) were found resistant, whereas all the accessions of *C. cuneatum*, *C. yamashitae* and *C. reticulatum* showed moderately susceptible to susceptible reaction to both the diseases.

Evaluation of wild derivative lines

A set of 193 wild derivative lines developed from interspecific cross between ICCV 96030 and *C. pin-*

Table 2. Disease response of wild *Cicer* spp. to Ascochyta blight (AB) and Botrytis grey mould (BGM).

Wild *Cicer* species	Disease Rating (1–9 Scale)[a]	
	AB	BGM
C. judaicum 182	2.0	3.0
C. judaicum 185	2.5	3.0
C. judaicum 185 A	6.0	7.0
C. judaicum 185 B	2.0	4.0
C. judaicum ILWC 95	4.0	4.0
C. judaicum ICC 17148	2.0	6.0
C. judaicum ILWC 61	3.0	2.0
C. judaicum ILWC 19-2	3.5	2.0
*C. bijugum*ILWC7/S-3	3.0	2.0
C. bijugumJM2103	5.0	NT[b]
C. bijugum 7	8.0	5.5
C. bijugum 194	8.0	4.3
C. bijugum201	7.0	6.0
C. pinnatifidum 199	2.0	3.0
C. pinnatifidum 188	3.0	2.0
C. pinnatifidum ILWC 9	5.0	2.0
*C. pinnatifidum*ILWC 212	5.0	3.0
*C. pinnatifidum*ILWC 9/S-1	2.0	2.0
C. reticulatum ILWC 129	9.0	6.0
C. reticulatum ILWC 257	8.0	7.0
C. reticulatum 2106A	6.0	NT
C. yamashitae ILWC 3	9.0	8.0
C. yamashitae JM 2021	9.0	9.0
C. yamashitae ICC 17157	7.0	8.0
C. echinospermum ILWC 39	3.0	2.0
C. echinospermum 204	6.0	6.0
C. echinospermum 35/S-1	1.0	1.0
C. cuneatum SL 157	7.0	NT

[a] Mean of two years (2009–2010 to 2010–2011).
[b] NT: not tested.

natifidum 188 were evaluated for AB and BGM resistance during 2008-09. Out of these, 62 lines with good growth habit and resistance for AB and BGM were further evaluated for two years (2009–2010 and 2010–2011) for confirmation of resistance. The mean response of these 62 lines to AB and BGM is given in Table 3. It was observed that some lines showed combined resistance to AB and BGM, while others to AB and BGM alone. Eleven lines, namely GL 29023, GL 29029, GL 29036, GL 29057, GL 29058, GL 29061, GL 29081, GL 29198, GL 29206, GL 29212 and GL 29243 were found resistant to both the diseases with consistent disease reactions. The wild parent *C. pinnatifidum* 188 was resistant to AB and BGM whereas the parent *C. arietinum* ICCV 96030 was susceptible.

Elucidation of *C. pinnatifidum* specific segments in derivative lines

One hundred and twenty SSR markers (Winter *et al*., 1999; Nayak *et al*., 2010) were deployed for marker analysis of four AB and BGM resistant derivative lines, GL 29029, GL 29206, GL 29212, GL 29081, and the parents *C. pinnatifidum* 188 and ICCV 96030. Out of 120, 12 primers generated *C. pinnatifidum* 188 specific bands in one or the other derivative lines (Figure 1). The SSR markers that flanked the *C. pinnatifidum* 188 specific segments in these derivative lines are listed in Table 4. As these lines showed AB and BGM resistance when phenotyped for resistance, the presence of the *C. pinnatifidum* specific segments in these lines further support the contribution of AB and BGM resistance in these derivative lines from the wild parent *C. pinnatifidum* 188.

Development of mapping populations

Of 11 derivative lines resistant to both AB and BGM, the four lines GL 29029, GL 29081, 29206 and 29212 were crossed with the susceptible parent BG 256 to develop F_2 populations which can be used for mapping AB and BGM resistance genes. The number of F_2 plants obtained from three of these crosses is given in Table 5. Parental polymorphism was identified in these three crosses by SSR marker and the markers showing polymorphism between parents are given in Figure 2 and Table 6.

Discussion

Annual wild species of chickpea are excellent sources of resistance to biotic and abiotic stress. Comprehensive screening of wild *Cicer* collections at inter-

Table 3. Disease reaction of derivative lines from cross ICCV 96030 × *Cicer pinnatifidum* 188.

S. No.	Derivative lines	Disease score[a]		S. No.	Derivative lines	Disease score[a]	
		AB[b]	BGM[b]			AB[b]	BGM[b]
1	GL 29006	5	2	34	GL 29096	7	9
2	GL 29008	5	6	35	GL 29098	2	6
3	GL 29009	6	2	36	GL 29099	5	6
4	GL 29013	3	5	37	GL 29198	3	3
5	GL 29015	6	5	38	GL 29204	4	3
6	GL 29017	9	4	39	GL 29206	2	2
7	GL 29020	4	6	40	GL 29212	3	2
8	GL 29021	5	5	41	GL 29213	6	3
9	GL 29022	5	6	42	GL 29224	2	5
10	GL 29023	3	2	43	GL 29243	3	3
11	GL 29026	3	5	44	GL 29248	9	4
12	GL 29029	2	3	45	GL 29267	7	6
13	GL 29031	7	3	46	GL 29269	3	5
14	GL 29034	6	5	47	GL 29272	6	5
15	GL 29036	2	3	48	GL 29004	8	5
16	GL 29045	6	5	49	GL 29018	-	4
17	GL 29046	4	6	50	GL 29019	3	3
18	GL 29047	5	6	51	GL 29039	3	5
19	GL 29052	5	2	52	GL 29042	3	3
20	GL 29057	3	3	53	GL 29066	4	4
21	GL 29058	3	3	54	GL 29067	3	4
22	GL 29059	4	3	55	GL 29068	6	5
23	GL 29061	3	2	56	GL 29069	6	4
24	GL 29063	5	2	57	GL 29071	6	2
25	GL 29064	5	2	58	GL 29072	8	6
26	GL 29065	5	3	59	GL 29221	3	4
27	GL 29076	6	3	60	GL 29232	3	3
28	GL 29079	3	6	59	GL 29244	5	4
29	GL 29080	3	6	60	GL 29088	4	3
30	GL 29081	3	2	61	GL 29095	2	4
31	GL 29083	6	5	62	GL 29278	3	6
33	GL 29093	7	7	63	ICCV 96030	9	9
				64	*C. pinnatifidum*	3	2

[a] 1–9 rating scale.
[b] Mean of three years.

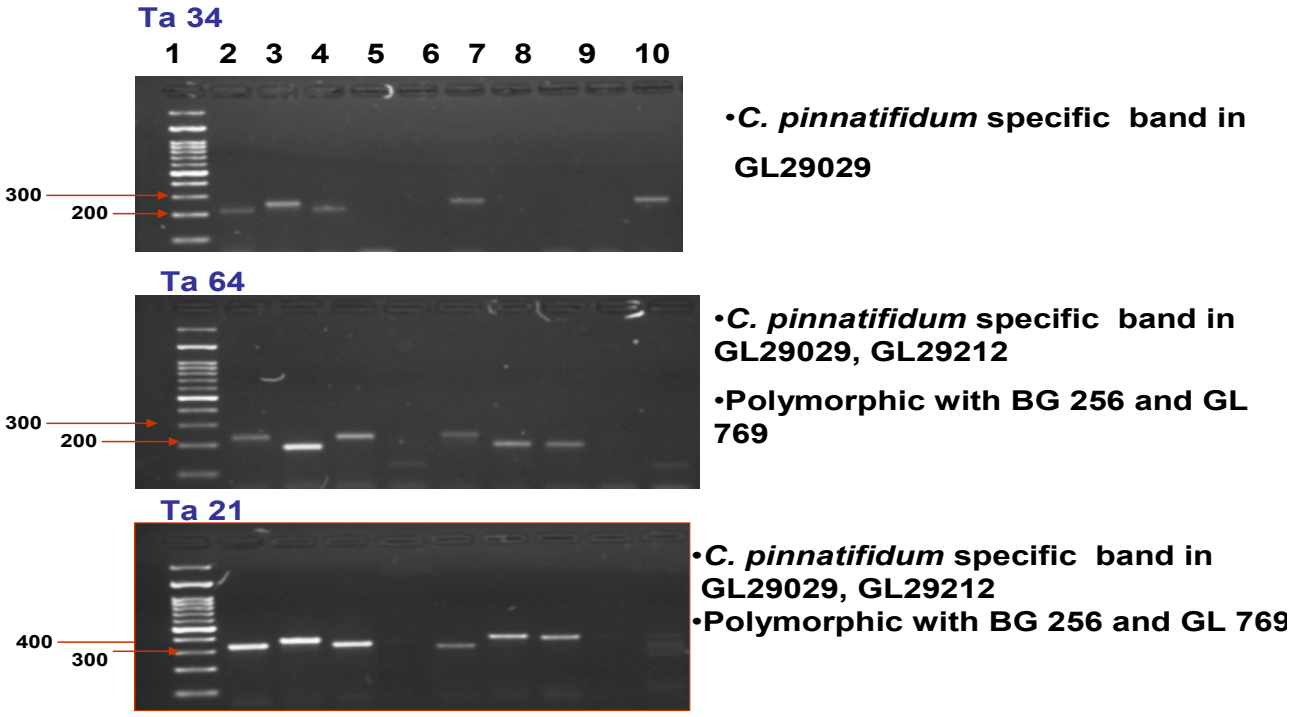

Figure 1. PCR amplified products with SSR primer Ta34 and Ta 64. Lane 1, Ladder 100 bp; 2, *C. pinnatifidum* 188; 3, ICCV 96030; 4, GL 29029; 5, GL29026; 6, GL29081; 7, GL 29081; 8, BG 256; 9, GL 769; 10, *C. pinnatifidum* 212. A) *C. pinnatifidum* specific band in GL29029; B) *C. pinnatifidum* specific band in GL29029 and GL29212; polymorphism between BG 256 and GL769.

Table 4. SSR primers displaying *Cicer pinnatifidum* 188 specific segments in four AB and BGM resistant derivative lines.

Derivative line	Primers
GL 29029	TS 72, ICCM 0160, psat_EST_189_01_1, TA 34, TA 64
GL 29206	TA 43, TA 21, CASTMS 24, ICCM 0068, TA 72, ICCM 0160,
GL 29212	TA 21, TA 64, TA 29, TS 72, ICCM 0160, psat_EST_189_01_1
GL 29081	TA 21, TA 110, TA 29, ICCM 0068, psat_ EST_189_01_1

Table 5. Populations developed from derivative lines and a susceptible chickpea (*C. arietinum*) cultivar.

Cross	F_1	F_2
BG 256 x GL 29206	19	218
BG 256 x GL 29212	32	165
BG 256 x GL 29081	22	152

national (ICARDA, ICRISAT) and national institutes has identified many accessions with disease and/or insect resistance (Croser *et al.*, 2003). At PAU, different accessions of *Cicer* have been extensively screened for AB and BGM resistance. Three accessions of *C. judaicum*, *C. pinnatifidum* and *C. echinospermum* showed resistance to AB and BGM. *C. judaicum* accessions 182, 185, 185A, and *C. pinnatifidum* accessions 188, ILWC212 had already been screened earlier (Singh *et al.*, 1991; Kaur *et al.*, 2007) and have maintained the resistance since 1990, hence have value as donors for introgression of AB and BGM resistance for enhancing the genetic diversity in chickpea. *C. pinnatifidum* 188 was used in interspecific hybridization (*C. arieti-*

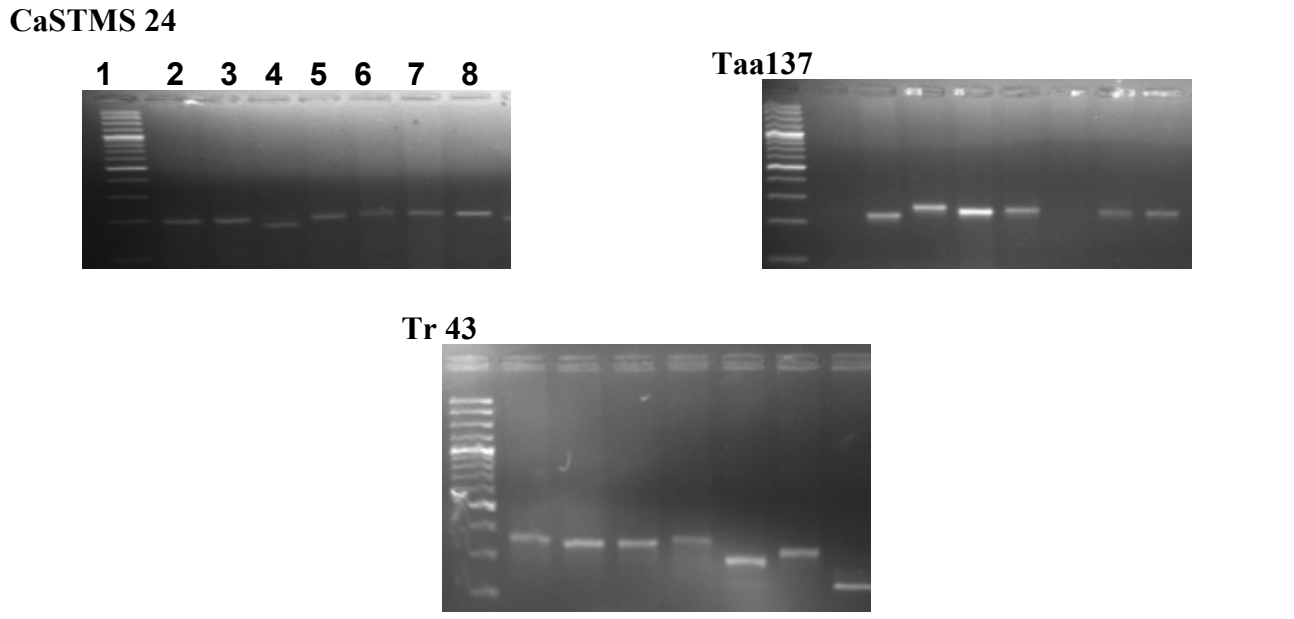

Figure 2. Parental polymorphism between BG256 and wild derivative lines. Lane 1, 100bp M; 2, *C. pinnatifidum* 188; 3; ICCV 96030; 4, GL 29029; 5, GL 29206; 6, GL29212; 7, GL29081; 8-BG 256.

Table 6. Primers showing polymorphism between chickpea parents involved in crosses.

Cross	Primers
BG 256 & GL 29029	TA 110, TA 2, TA 72, TA 203, TA 47, CASTMS 24, TAA 137, TA 64, TA 43, TA 5, TS 12, TR 43, CASTMS 23, TS 29, ICCM 0068, ICCM 0178, ICCM 0242a, TR 20
BG 256 & GL 29206	TA 2, TR 43, CASTMS 24, TA 110, TA 72, TA 203, TAA 137, TA 64, TA 47, TA 43, TA 28, TS 29, ICCM 0068, ICCM 0242a, TS 12
BG 256 & GL 29212	TA 72, TA 203, CASTMS 24, TA 64, TA 43, ICCM 0178, TS 12, TA 110, TA 2, TR 43, TA 28, TS 29

num ICCV 96030 × *C. pinnatifidum* 188) and 62 derivative lines developed from the cross have high level of resistance to AB and BGM and fairly good agronomic characters. Further evaluation of these lines for other biotic and abiotic characters would be desirable to determine their use as donors for further improvement of chickpea. Interspecific hybridization using *C. pinnatifidum* has been reported to produce fertile hybrids when using growth regulators, however, the percentage seed set was very low (Verma *et al*., 1990). An alternate to this, embryo rescue technique, has been deployed for obtaining viable hybrids from interspecific hybridization (Singh and Singh, 1989; Badami *et al*., 1997; Van Dorrestein *et al*., 1998; Mallikarjuna, 1999, 2001; Stamigna *et al*., 2000). Application of embryo rescue technique is cumbersome and needs a well established tissue culture laboratory. Despite the low success rate of developing F_1 hybrids using conventional methods or the requirement for and difficulties in embryo rescue techniques, developing interspecific hybrids from the *C. pinnatifidum* appear to be a useful strategy for exploitation of *C. pinnatifidum* spp. to improve disease resistance in cultivated chickpea and to enhance the genetic diversity in breeding programs. The resistant derivative lines generated can serve as good pre-breeding material. The mapping populations developed from three derivative lines and the polymorphic markers identified in the present study will be useful for identification of markers linked with AB and BGM resistance which can further assist in marker assisted selection for resistance breeding.

Acknowledgement

We are grateful to Department of Biotechnology, Ministry of Science & Technology, India for giving us financial assistance to run an ad hoc project entitled "Interspecific gene transfer and molecular tagging of Botrytis Gray Mould resistance genes in chickpea", a part of which is included in the present study.

Literature cited

Abbo S., J. Berger and N.C. Turner, 2003. Evolution of cultivated chickpea: four bottlenecks limit diversity and constrain adaptation. *Functional Plant Biology* 30, 1081–1087.

Badami P.S., N. Mallikarjuna and J.P. Moss, 1997. Interspecific hybridization between *Cicer arietinum* and *C. pinnatifidum*. *Plant Breeding* 116, 393–395.

Choumane W., P. Winter, F. Weigand and G. Kahl, 2000. Conservation and variability of sequence - tagged microsatellite sites (STMS) from chickpea (*Cicer arietinum* L.) within the genus *Cicer*. *Theorotical and Applied Genetics* 101, 269–278.

Croser J.S., F. Ahmad, H.J. Clarke and K.H.M. Siddique, 2003. Utilization of wild *Cicer* in chickpea improvement- progress, constrains and prospects. *Australian Journal of Agricultural Research* 54, 429–444.

Gargav S. and P.M. Gaur, 2001. Phylogenetic relationship of perennial chickpea species *Cicer microphyllum* (Benth.) with its annual relatives as revealed by allozyme Polymorphism. *Indian Journal of Genetics* 61, 218–222.

Gurha S.N., G. Singh and Y.R. Sharma, 2003. Diseases of chickpea and their management. In: *Chickpea Research in India* (M. Ali, S. Kumar, N.B. Singh, ed.), Indian Institute of Pulses Research, Kanpur, India, 195–227.

Haware M.P., 1998. Diseases of chickpea. In: *The Pathology of Food and Pasture Legumes*. (D.J. Allan, J.M. Lenne, ed.), ICARDA, CAB International: Wallingford, UK, 473–516.

Kaur L., J.S. Sandhu and S.K. Gupta, 2007. Inter and intra accession variation for resistance to botrytis grey mould of chickpea in wild *Cicer* species. *Indian Journal of Agricultural Sciences* 77, 786–788.

Kumar S., S. Gupta, S. Chandra and B.B. Singh, 2004. How wide is the genetic base of pulse crops? In: *Pulses in New Perspective* (M. Ali, B.B. Singh, S. Kumar Vishwadhar, ed.), IIPR, Kanpur, India, 211–221.

Kumar J., R.R. Mir, N. Kumar, A. Kumar, A. Mohan, K.V. Prabhu, H.S. Balyan and P.K. Gupta, 2010. Marker-assisted selection for pre-harvest sprouting tolerance and leaf rust resistance in bread wheat. *Plant Breeding* 33, 145–217.

Mallikarjuna N. 1999. Ovule and embryo culture to obtain hybrids from interspecific incompatible pollination in chickpea. *Euphytica* 110, 1–6.

Mallikarjuna N., 2001. Prospects of using *Cicer canariense* for chickpea improvement. *International Chickpea and Pigeonpea Newsletter* 8, 23–24.

Nayak S.N., H. Zhu, N. Varghese, S. Datta, H.K. Choi, R. Horres, R. Jungling, J. Singh, P.B. Kavi Kishor, S. Sivaramakrishnan, D.A. Hoisington, G. Kahl, P. Winter, D. R. Cook and R.K Varshney, 2010. Integration of novel SSR and gene-based marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. *Theoretical and Applied Genetics* 120, 1415–1441.

Saghai-Maroof M.A., K.M. Soliman., R.A. Jorgensen and R.W. Allard, 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Science* 81, 8014–8018.

Singh G., 1990. Identification and designation of physiologic races of *Ascochyta rabiei* in India. *Indian Phytopathology* 43, 48–52.

Singh G. and L.K. Bhan, 1986. Physiological Races of *Botrytis cinerea* causing gray mold of chickpea. *Plant Disease Research* 1, 69–74.

Singh K.B., L. Holly and G. Bejiga (ed.), 1991. Catalogue of kabuli chickpea germplasm. In: *An Evaluation Report of Winter Sown Kabuli Chickpea Landraces, Breeding Lines and Wild Cicer Species.* ICARDA publishers, Aleppo, Syria, 393–397.

Singh N.P. 2012. Project Co-coordinator's report, All India Coordinated Research Project on Chickpea, Indian Institute of Pulses Research, Kanpur, India.

Singh R.P. and B.D. Singh, 1989. Recovery of rare interspecific hybrids of gram *Cicer arietinum* x *C. cuneatum* (L.) through tissue culture. *Current Science* 58, 874–876.

Stamigna C., P. Crinò and F. Saccardo, 2000. Wild relatives of chickpea: Multiple disease resistance and problems to introgression in the cultigens. *Journal of Genetics and Plant Breeding* 54, 213–219.

Van Dorrestein B., M. Baum and R.S. Malhotra, 1998. Interspecific hybridization between cultivated chickpea (*Cicer arietinum* L.) and the wild annual species *C. judaicum* Boiss, *C. pinnatifidum* Jaub. and Sp. and *C.bijugum* KH. Rech. In: *Opportunities for High Quality, Healthy and Added Value Crops to Meet European Demands*. Proceedings of the third European conference on grain legumes (AEP) (European Association for Grain Legume Research, ed.). Valladolid, Spain, 362–363.

Verma M.M., J.S. Sandhu, H.S. Brar and J.S. Brar, 1990. Crossability studies in different species of *Cicer* (L.). *Crop Improvement* 17, 179–181.

Winter P., T. Plaff, S.M. Udupa, B. Huttel, P.C. Sharma, S. Sahi, R. Arreguin-Espinoza, F. Weigand, F.J. Muehlbauer and G. Kahl, 1999. Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Molecular and General Genetics* 262, 90–101.

Zohary D. and M. Hopf, 2000. Pulses. In: *Domestication of Plants in the Old World. The Origin and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley*, Oxford University Press, New York, NY, USA, 108–111.

Accepted for publication: January 22, 2013